

## **REMARKS**

### **Status**

Claims 87-140 were at issue in the Office Action mailed on July 9, 2008. This response does not cancel or add any claims. Accordingly, it is claims 87-140 which are at issue in this response.

### **The Office Action**

In the Office Action mailed July 9, 2008, claims 87-140 were rejected. Specifically, claims 87-88, 92-93, 97-100, 107, 109, 112-113, 115, 117-118, 120-129, 137 and 140 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lea et al. (US 5,428,451) in view of Hansen et al. (EP 1 180 675).

Claims 89-90 and 95-96 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lea et al. '451, Hansen et al. '675 as applied to claim 87 and further in view of Griffiths (US 2002/0119459).

Claims 91, 102, 107 and 130 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lea et al. '451, Hansen et al. '675 as applied to claims 87-88 and further in view of Baer et al. (US 5,547,849).

Claims 92-93, 103, 110-111 and 114 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lea et al. '451, Hansen et al. '675 as applied to claims 87-88 and further in view of Singer et al. (US 5,728,527).

Claims 92 and 94 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lea et al. '451, Hansen et al. '675 as applied to claims 87-88 and further in view of Connors et al. (US 5,726,009).

Claims 101, 108 and 110 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lea et al. '451, Hansen et al. '675 as applied to claims 87-88 and further in view of Singer et al. (US 5,728,527).

Claims 102, 104, 107 and 131-132 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lea et al. '451, Hansen et al. '675 as applied to claim 87 and further in view of Riabowol (US 5,877,161).

Claims 105, 107 and 135 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lea et al. '451, Hansen et al. '675 as applied to claim 87 and further in view of Draetta et al. (US 5,691,147).

Claims 106, 107 and 136 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lea et al. '451, Hansen et al. '675 as applied to claim 87 and further in view of Bitler et al. (US 6,379,882).

Claims 116, 118, 138 and 139 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lea et al. '451, Hansen et al. '675 as applied to claims 87 and 117 and further in view of Bobrow et al. (US 2003/0110846).

Claim 119 was rejected under 35 U.S.C. §103(a) as being unpatentable over Lea et al. '451, Hansen et al. '675 as applied to claim 87 and further in view of Mathies et al. (US 6,100,535).

Claims 102, 104-105, 107, 131 and 133 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lea et al. '451, Hansen et al. '675 as applied to claim 87 and further in view of Sherley et al. (US 5,741,646).

And finally, claims 102, 104-105, 107 and 134 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lea et al. '451, Hansen et al. '675 as applied to claim 87 and further in view of Harvey et al. (US 5,344,760).

Applicant thanks the Examiner for the Office Action, for the search and for the thorough explanation of the basis of the rejections.

### **The Present Invention**

The method according to the present invention relates to the detection of particles, wherein the particles are identified due to labelling of analytes on the particles, wherein said analytes are present in a low number of detectable positions. Thereby the total amount of signal from one particle is lower as compared to signals from for example the same type of particle being labelled on other analytes, said other analytes being present in a much higher number of detectable positions on each particle.

Furthermore, the present invention relates to a detection method, wherein a volume of liquid material comprising a mixture of sample and reagent material is exposed to an array of detection elements at standstill conditions.

Accordingly, the present invention relates to a combination of:

- the labelling method of small amount analytes, and
- the detection method

### **Different types of labelling of biological cells**

A biological cell consists of a cell membrane defining the cell and in the cell are located the cell nucleus as well as other organelles. Counting biological cells or performing other studies of biological cells mostly requires that the cells are visualised through some means. A common

form of visualising a biological cell is through labelling using a fluorophore or a chromophore. The label is either by itself or through a molecule to which it is attached capable of binding to a specific part of the cell. In particular, if the purpose of counting is to count all cells present, the label is often directed to the cell core, wherein the DNA is positioned, since more than  $10^9$  detectable positions are available for binding to a cell core label.

However, for other purposes it is only relevant to count cells presenting a specific marker, such as the CD4 marker. In such situations the label is targeted towards that specific marker, and the only detectable positions available for the label is the positions of the specific marker, typically as proteins being positioned on the surface of the cell. Thereby, fewer positions are available to be labelled as compared to the cell core labelling.

Obviously, the amount of signal obtainable from a cell being labelled at the DNA in the cell is much higher than the total amount of signal from a cell being labelled at much fewer positions.

An analogy is positioning of Christmas candles on a Christmas tree. If the candles are arranged on every little branch and side branch many more candles may be arranged on the tree as compared to the situation that candles are only placed on branches 5 cm from where they branch off from the main stem of the tree. In the latter situation only a few candles may be arranged on the tree, and therefore the total light signal from the tree is much lower than in the former situation.

Since it is the amount of signal that is captured when counting or otherwise assessing cells, it is of great importance whether the type of detection system is capable of “seeing” the cell, i.e. whether the amount of signal is high enough to allow the detection system to “see” the cell.

Therefore, since a biological cell may be labelled at different positions, and since the number of positions differ depending on the analyte to be labelled, then the amount of signal arising from a particular labelling differs from analyte to analyte. For example a white blood cell may be cell core labelled, whereby the analyte is the DNA, as is the case in Lea et al. or may be labelled on a specific marker on the cell surface as is the case in the present invention, and the amount of signal differs by a factor 1000. Thus, the mere fact that a specific cell is used in an assay does not provide the reader with any information about the analyte assessed.

### **Detection method**

Labelled cells may be detected by different detection methods, in particularly depending on the type of labelling.

Hydrodynamic detection methods, also denoted flow systems or flow cytometry, are well known in the art. Normally such detection methods work according to the following principle:

The sample is transported through an interrogation point and for accurate data collection, it is important that particles or cells are passed through the beam one at a time, i.e. the flow is arranged so that there is a large separation between cells relative to their diameter. Most flow cytometers accomplish this by injecting the sample stream containing the cells into a flowing stream of sheath fluid, where the sample stream is compressed to roughly one cell in diameter for hydrodynamic focusing.

Lea et al. discloses a variant of the conventional flow system, in that the fluid stream of Lea et al. does not have a sheath fluid. However, the principle of Lea et al. is still a flow system and if the fluid stream is stopped then the principle of Lea et al. does not work.

The speed of the flow passing the detector may be controlled so that a distance between each cell and the next cell is ensured.

Therefore, even if the speed is reduced, no skilled person, while using flow cytometry and requiring dynamic scanning would make the sample to stand still because that would disrupt the working principle of flow cytometry as it is based on hydrodynamic scanning of one particle at a time. Hence, standstill sample assessment is contrary to the scope of flow cytometry technique.

A completely different method is a method, wherein the cells are fixed on a slide, such as the method used in for example Singer et al. (discussed below) and Bobrow et al. (discussed below), or wherein a solid tissue volume is studied in a microscope, such as the method in for example Connors et al. (discussed below).

It goes without saying that a flow cytometry principle cannot be combined with a system using cells fixed on a slide.

Another completely different method is disclosed in Baer et al. wherein a laser is scanning a tube comprising a sample.

Again it goes without saying that a flow cytometry principle cannot be combined with the principle in Baer et al. since using a scanning laser in combination with a flow system would result in parts of the sample not being analysed, as discussed below.

Furthermore, the amount of signal necessary to provide the analysis of the cell differs from system to system. Accordingly, although a cell being labelled on a specific marker is visible in one system it may not be visible in another system, if said other system requires more signal to “see” the labelled cell.

Thus, although cells have been labelled on low amount analytes in the prior art and detected in methods and systems completely different from the method of the present invention,

then the skilled person has no expectation of success as to whether the same cell being labelled on a corresponding analyte is assessable by the present invention.

**The Rejections under 35 U.S.C. §103**

All of the claims have been rejected under 35 U.S.C. §103(a) as being unpatentable over Lea et al. (US 5,428,451) in view of Hansen et al. (EP 1 180 675) as the core two references. Given the general inapplicability of the Lea reference in combination with the Hansen reference as discussed in detail below, further rejections under 35 U.S.C. §103 are inappropriate. In addition, Applicant will further discuss the general inapplicability of each of the specific rejections hereinbelow.

A. The invention is non-obvious in view of Lea et al. (US 5,428,451) combined with Hansen et al. (EP 1180675)

The technical problem to be solved by the present invention is “How to measure labelled particles with a low amount of a visual label”, which is well correlated with the object stated in the description of the present invention on page 2, lines 13-15:

It is an object of the present invention to provide an alternative method for the assessment of properties related to particles based on staining of analytes which are several orders of magnitude less abundant than DNA monomers.

Furthermore, the present invention relates to assessment of a volume of sample comprising the particles having the analytes, wherein the volume of sample is at standstill conditions.

As discussed above the method according to Lea et al. relates to a flow system. The flow cytometry method of Lea et al. may introduce errors that are inherent in the assessment of particles caused by variation in flow conditions, particularly when an assessment of a property is a volume related property such as the counting of particles in a volume of sample. Although,

Lea et al. mentions that if the particle stream is found to be moving too fast for accurate counting, the speed may be controlled (col. 3, lines 20-30), but it should be noted here the method according to Lea et al. does not function if the speed is reduced to zero, as discussed above.

Furthermore, the Examiner co-related blood cells of Lea et al. with preferred form of particles, i.e. blood cells of the present application, and concluded that Lea's reference teaches assessing particles having less than  $1 \times 10^6$  detectable positions (Page 4, item 7 of the Action).

However, the applicant firmly traverses this assumption because it is to be noted that Lea et al. uses a DNA (cell core) labelling, i.e. acridine orange (col. 5, line 35 of Lea et al.) which as discussed above produces a much higher number of (approximately  $10^9$ ) detectable positions for the same blood cell as opposed to that obtained from surface binding, which will produce less than  $1 \times 10^6$  detectable positions. Therefore, using the same cell is certainly not an indication of available active detectable positions being labelled.

The Examiner in item 7 further pointed out that the particles are bound to reagents in the form of superparamagnetic beads or via a sandwich complex (col. 4, line 25 – col. 5, line 5). However, the cited art further mentions that the magnetic particles are typically attached to selected cells, which after processing, may be conveniently lysed and only the nuclei which have previously been stained by a fluorescent dye are counted (col. 4, lines 59-62). This clearly indicates that before the use of flow cytometry technique, the Lea's method is based on breaking the cellular membrane and creating a lysate, followed by counting only the stained nuclei (cell core), thereby indicating production of high number of detectable positions, as described above, and hence making the cited art principally different from the present invention.



The person skilled in the art would hence not have a reasonable expectation of success if he or she should consider attempting to make an assessment of quantitative and qualitative parameters of particles with a low amount of analyte detectable positions using the method according to Lea et al.

Consequently, from Lea's teachings, a skilled person would be restricted to a hydrodynamic technique and with particles having a high number of detectable positions.

Hansen et al. discloses a method and system for assessment of properties of particles in a liquid sample. Hansen et al. also relates to assessing by staining the particles with stains which are known to stain DNA monomers (about  $10^9$  available detectable positions), for example see the Examples describing labelling by use of fluorochrome binding to DNA, such as Propidium Iodide and Ethidium Bromide. Accordingly, Hansen et al. discloses methods for assessment based on staining abundant molecules in the cells. Under such conditions, the signal accumulated from each cell is relatively high and the signal to noise ratio is correspondingly low so that distinction between signal from particles and background is facilitated. Although, Hansen et al. teaches assessment of particles under stand still positions (Para 70), there is no teaching in Hansen et al. to solve the technical problem of "How to measure labelled particles with a low amount of a visual label" less than  $1 \times 10^6$ .

In combination with Lea et al., it is observed that the cited arts are contradictory in nature, if combined, because bringing the speed of a flow cytometer to zero is not in compliance with the working principle of flow cytometry because in a stand still particle position, Lea's teaching would seize to operate in the area of flow cytometry.

Furthermore, both Lea et al. and Hansen et al. disclose labelling of DNA, i.e. a cell core labelling.

Accordingly, neither Lea et al. nor Hansen et al. discloses the step in claim 87:

mixing the liquid material with at least one reagent material, said reagent material at least comprising a first targeting species capable of selectively and directly binding to an analyte position of said species of analytes, said species of analytes having an amount of less than  $1 \times 10^6$  analyte detectable positions and a labelling agent....

Thus, it may be concluded that the two references cannot be combined since they are contradictory in nature, and furthermore, the combination does not lead to the present invention since neither references discloses the step of labelling low amount analytes.

Claim 87 and all claims dependent thereon are hence non-obvious in view of Lea et al. combined with Hansen et al.

The remaining references are cited with respect to subclaims, however in the following arguments as to their lack of relevance for claim 87 is provided.

- B. The invention is non-obvious over combination of Lea et al. (US 5,428,451), Hansen et al. (EP 1180675) and Griffiths (US 2002/0119459)

Griffiths describes a method for sorting genetic elements encoding a gene product having a desired activity. This method is based on change in the optical property (change in fluorescence) of the genetic elements. The sorting is performed using a fluorescence activated cell sorter (FACS) or its equivalent (claim 37). Therefore, Griffiths applies a flow technique on one microcapsule at a time, wherein the microcapsules one-by-one in flow motion are sorted based on the optical property obtained from the microcapsules. The flow cytometry technique applied in Griffiths' teaching is contrary to the scope and working principle of the present invention. Therefore, if FACS teaching of Griffiths is combined with Lea's reference, the combined teachings would still be in the area of hydrodynamic technique, thereby not providing

any hints to a skilled person regarding standstill assessment of particles because the skilled person would still be restricted to employing a flow cytometry technique.

When further combined with Hansen et al., the combined hydrodynamic teachings of Lea et al. and Griffiths are contradictory to hydrostatic teaching of Hansen et al.

Therefore, there is no expectation of success in combination of the teachings and suggestions of Lea et al., Hansen et al. in view of Griffiths, as advanced by the Examiner, and any attempt to combine the teaching arises from using applicant's invention as a template through a hindsight reconstruction of applicant's claims.

Consequently, claims 89, 90, and 95-96, when read with claim 87 are hence non-obvious in view of Lea et al. combined with Hansen et al. further combined with Griffiths.

C. Invention is non-obvious in view of Lea et al. and Hansen et al. and Baer et al. (US 5,547,849)

Baer et al. discloses analysis of cells using yet another detection method as compared to Lea et al. and Hansen et al. In the method according to Baer et al., a highly focused laser beam is scanned over a capillary tube constituting the sample compartment. The combined effect of the high intensity of the highly focused laser beam and the option of using relatively long exposure times, allows for an assessment of qualitative and quantitative parameters of particles with a low number of detectable positions.

In Lea et al., the sample flows through the sample compartment. When the sample is flowing during the optical inspection, an image of the entire inspected section of the sample compartment must be taken instantaneously in order to probe the entire sample volume. If a highly focused laser beam is scanned over the sample compartment while the sample is flowing, there will unavoidably be sections of the sample, that are not exposed to the laser beam and the

optical inspection will provide an incomplete detection of the particles in the sample. The method according to Lea et al. is hence incompatible with the method according to Baer et al.

The shortcomings of Lea et al. with respect to the assessment of quantitative and qualitative parameters of particles with less  $10^6$  analyte detectable positions particles cannot be corrected by modifying the method using features of a system for scanning a highly focused laser beam over the sample instead of acquiring an image of the sample in the sample compartment.

The mere fact, that Baer et al. describes that it is possible to determine the content of particles by using a scanning laser does not provide the skilled person with any expectation of success with respect to the possibility of assessing particles based on labelled analytes having less than  $1 \times 10^6$  positions in a completely different system. The Examiner tends to believe that if a given particle labelled at given analytes is detectable in one type of system, then it is possible to detect the same particle in any other detection system and method. This is an unallowable generalisation and simplification of the invention.

Furthermore, it is simply not possible to construct the method according to the present invention by arbitrarily choosing from the combined teachings of Lea et al. and Hansen et al. and Baer et al. without the use of impermissible hindsight.

Claim 87 and all claims dependent thereon are hence inventive in view of Lea et al. combined with Hansen et al. further combined with Baer et al.

D. Invention is non-obvious in view of Lea et al. and Hansen et al. and Singer et al. (US 5,728,527)

In Singer et al. the assessment of the species that is detected is based on taking photomicrographs (col. 10, lines 22-29) of stained cells fixated on e.g. a glass slide (col. 9, line

65 – col. 10, line 3 and in col. 12, line 60 – col. 13, line 2). The cells that are to be analyzed are actually grown on glass cover slips (col. 11, lines 33-39).

Lea et al. on the other hand relates to a method for the detection of particles in a liquid sample and hence the detection methods of Lea et al. and Singer et al. cannot be combined.

The mere fact, that Singer et al. describes that it is possible to determine the content of particles fixated on a glass slide does not provide the skilled person with any expectation of success with respect to the possibility of assessing particles in a liquid sample based on labelled analytes having less than  $1 \times 10^6$  positions in a completely different system.

The Examiner tends to believe that if a given particle labelled at given analytes is detectable in one type of system, then it is possible to detect the same particle in any other detection system and method. This is an unallowable generalisation and simplification of the invention.

Claim 87 and all claims dependent thereon are hence inventive in view of Lea et al. combined with Hansen et al. further combined with Singer et al.

E. Invention is non-obvious in view of Lea et al. and Hansen et al. and Connors et al. (US 5,726,009)

Connors et al. relates to a method of using an in vitro culture system to measure the cell proliferation and cell viability of human tissues. Connors et al. measures number of cells in a histocultured tissue sample by incorporating a DNA-synthesis marker into the proliferating cells. That is, the number of proliferating cells in the tissue is indicated by the number of cells in the histocultured tissue sample having metabolically incorporated the DNA-synthesis marker into the cellular DNA during cell division associated with cell proliferation.

As described in col. 3, lines 45-67 the three dimensional structure of the tissue sample is maintained during culturing and subsequent optical characterization. The method according to Connors et al. is hence fundamentally different from the method according to Lea et al. wherein a liquid sample flows by the detection element.

The teachings of Lea et al. and Connors et al. can hence not be combined in a reasonable manner.

In relation to the magnification used in the optical setup, Connors et al. describes in col. 7, lines 35-37 that a magnification of 200:1 is used. There is hence no disclosed in Connors et al. of a method wherein a magnification below 20:1 is used.

The mere fact, that Connors et al. describes that it is possible to determine the content of particles in a tissue sample while maintaining the structure of the tissue does not provide the skilled person with any expectation of success with respect to the possibility of assessing particles in a liquid sample based on labelled analytes having less than  $1 \times 10^6$  positions in a completely different system.

The Examiner tends to believe that if a given particle labelled at given analytes is detectable in one type of system, then it is possible to detect the same particle in any other detection system and method. This is an unallowable generalisation and simplification of the invention.

Claim 87 and all claims dependent thereon are hence inventive in view of Lea et al. combined with Hansen et al. and further combined with Connors et al.

F. The invention is non-obvious over combination of Lea et al. and Hansen et al. and Riabowol (US 5,877,161)

Riabowol relates to cyclin D1 as a regulator of cell proliferation. The scope of Riabowol is to regulate cell growth using cyclin D1 rather than the counting method *per se*. Riabowol, therefore, utilizes commonly available techniques, namely autoradiography or fluorography to identify the growth. The labels used in Riabowol's method include both radioactive and fluorescent dyes.

When cells are studied they are fixated on a slide, see col. 6, lines 43-48.

As discussed in relation to Singer et al., the mere fact that Riabowol describes that it is possible to visualise cells fixated on a slide does not provide the skilled person with any expectation of success with respect to the possibility of assessing particles in a liquid sample based on labelled analytes having less than  $1 \times 10^6$  positions in a completely different system.

Claims 102, 104, 107, and 131-132, when read with claim 87 are hence non-obvious in view of Lea et al. and Hansen et al. combined with Riabowol.

G. Invention is non-obvious in view of Lea et al. and Hansen et al. and Draetta et al. (US 5,691,147)

Draetta et al. relates to the discovery of novel proteins capable of binding the human cyclin dependent kinase 4 (CDK4). In col. 25, line 67 to col. 26, line 35 is briefly described the detection of lesions in genes encoding a protein binding to the CDK4.

It is mentioned that the CDK4 binding protein can be detected in an immunoassay (col. 26, lines 34-35). But Draetta et al. provides no specific information relating to the method for the optical detection of these species.

The mere fact, that Draetta et al. describes that it is possible to determine the content of particles using for instance an immunoassay does not provide the skilled person with any

expectation of success with respect to the possibility of assessing particles in a liquid sample based on labelled analytes having less than  $1 \times 10^6$  positions with the method according to the amended claims of the current application.

The combination of Lea et al. and Hansen et al. does not lead to the invention claimed in claim 87 and a further combination with Draetta et al. does not lead to the invention either, since there is no guidance in Draetta et al. of how to analyse the cells.

The Examiner tends to believe that if a given particle labelled at given analytes is detectable in one type of system, then it is possible to detect the same particle in any other detection system and method. This is an unallowable generalisation and simplification of the invention.

Claim 87 and all claims dependent thereon are hence inventive in view of Lea et al. combined with Hansen et al. further combined with Draetta et al. (US 5,691,147)

H. Invention is non-obvious in view of Lea et al. and Hansen et al. and Bitler et al. (US 6,379,882)

Bitler et al. describes a method for selecting therapeutic agents for in vivo treatment. A number of different methods for detecting fluorescently stained cells are briefly mentioned: flow-cytometry (col. 12, line 48), microscopy (e.g. col. 13, lines 3-4; col. 13, lines 9-12; and col. 14, line 5).

However, since the combination of Lea et al. and Hansen et al. cannot be combined then addition of a further reference discussing yet other methods of detecting cells, methods different from the claimed invention, does not provide a combination of references that leads to the present invention.



As discussed several times above the Examiner tends to believe that if a given particle labelled at given analytes is detectable in one type of system, then it is possible to detect the same particle in any other detection system and method. This is an unallowable generalisation and simplification of the invention.

Claim 87 and all claims dependent thereon are hence inventive in view of Lea et al. combined with Hansen et al. further combined with Bitler et al. (US 6,379,882).

I. The invention is non-obvious over combination of Lea et al. and Hansen et al. and further combined with Bobrow et al. (US 2003/0110846)

Bobrow et al. provides an analytical system relying on a method for enhancing the sensitivity of array-based analytical assay systems, i.e. Bobrow et al. relates to amplification of signals from specific binding pairs. The samples are fixed on slides, see the Examples, and afterwards scanned.

The specific binding pair includes DNA, cDNA, RNA, oligonucleotides, and combinations thereof.

The detection method of Bobrow et al. is a non-flow non-liquid based method, which is in contradiction to the principle of Lea et al.'s flow cytometry technique and to the liquid method of Hansen et al., and furthermore, the detection method of Bobrow et al. differs from the method of the present invention.

Accordingly, since the combination of Lea et al. and Hansen et al. cannot be combined then addition of a further reference discussing yet another method of detecting cells, a method different from the claimed invention, does not provide a combination of references that leads to the present invention.

Claims 116, 118, 138 and 139, when read with claim 87 are hence non-obvious in view of combined teachings of Lea et al. and Hansen et al. and Bobrow et al.

J. Invention is non-obvious in view of Lea et al. and Hansen et al. and Mathies et al. (US 6,100,535)

Mathies et al. describes a system for detecting electrophoretic separations in capillary tubes.

When using a system for electrophoretic separation to analyse samples containing variously-sized particles (such as stained DNA fragments), the particles are spatially separated by an electrical field applied over the sample.

When subjecting a sample containing a number of differently-sized DNA fragments stained with a fluorescent dye to an electrophoretic separation, the DNA fragments of same size will form a coherent population at a location in the capillary tube determined by the fragment size. Fluorescence emitted from the stain molecules when exposing the capillary tubes to an excitation light source is seen as bands.

The content of the examined sample is identified by comparing the position of the bands of the sample under analysis with the location of the bands from stained DNA fragments in a reference sample.

The principle of electrophoretic separation requires a non-flow, non-liquid system in order for the various bands to form. The system according to Mathies et al. hence relates to a measurement technique that is incompatible with the method according to Lea et al. as well as to Hansen et al. and it is impossible to perform the method of Mathies et al. in the detection methods of Lea et al. or Hansen et al.

Thus, since the combination of Lea et al. and Hansen et al. cannot be combined then addition of a further reference discussing yet another detection method, a method different from the claimed invention, does not provide a combination of references that leads to the present invention.

Claim 87 and all claims dependent thereon are hence inventive in view of Lea et al. combined with Hansen et al. further combined with Mathies et al.

K. The invention is non-obvious over combination of Lea et al. and Hansen et al. and Sherley et al. (US 5,741,646)

Sherley et al. relates to a method for determining the effect of a substance on cell growth kinetics. Sherley et al. gives a general reference to the use of bromodeoxyuridine-Hoechst dye fluorescence quench procedure to visualize and quantify the daughter cell products of induced stem cell division. It is to be observed here that Sherley et al. does not give information about any particular assessment technique except for the use of a fluorescent dye, and Bromodeoxyuridine (5-bromo-2-deoxyuridine, BrdU) dye is commonly used in the detection of proliferating cells in living tissues. BrdU is usually incorporated into the newly synthesized DNA of replicating cells, thereby, making it a cell core binding and leading to high number of detectable positions, as already mentioned before.

Furthermore, Sherley et al. does not disclose any detection method.

Accordingly, Sherley et al. refers to a cell core labelling method without disclosing any method for detection, and therefore, since Sherley et al. does not include the step of labelling analytes being present in an amount of less than  $10^6$  per particle, then the teaching of Lea et al and Hansen et al. is not supplemented by the teaching of Sherley et al.

Sherley et al. uses p53 positive and negative cells, however Sherley et al. does neither label nor detect p53 in or on the cells.

Claims 102, 104-105, 107, 131, and 133, when read with claim 87 are hence non-obvious in view of Lea et al. combined with Hansen et al. further combined with Sherley et al.

L. The invention is non-obvious over combination of Lea et al. and Hansen et al. and Harvey et al. (US 5,344,760)

Harvey et al. relates to a diagnostic/prognostic method for squamous cell carcinoma or urinary tract cancer comprising detecting and/or quantifying in body fluids or tissue extracts, such as a mammalian urine or bladder wash sample a portion of the epidermal growth factor (EGFr) which comprises substantially the EGFr ectodomain and which has a molecular weight in the range of from about 90 kilodaltons (kd) to about 115 kd.

It is described at col. 6, lines 59-62 that malignant tumors *release* a higher level of the EGFr ectodomain protein into body fluids than do benign tumors or normal tissue. Accordingly, since the EGFr is *released* into the body fluids, then the EGFr detected has no association with cells or other particles during detection. At col. 7, lines 24-44 it is described how the EGFr proteins are detected, all of the methods being conventional methods for detecting free proteins and protein fragments.

Accordingly, Harvey et al. does not disclose any detection of particles, and therefore Harvey et al. does not disclose any detection of particles having a low amount of analytes to be labelled.

Thus, since the combination of Lea et al. and Hansen et al. cannot be combined then addition of a further reference discussing detection of substances not being particles, does not provide a combination of references that leads to the present invention.

Claims 102, 104-105, 107 and 134, when read with claim 87 are hence non-obvious in view of Lea et al. combined with Hansen et al. further combined with Harvey et al.

**Conclusion**

In view of the amendments and remarks presented herein, Applicant respectfully submits that all rejections have been overcome and the application is in condition for allowance. Therefore, Applicant respectfully requests that the rejections to claims 87-140 be withdrawn and the application moved towards allowance. Should the Examiner have any questions, comments or suggestions which would place the application in still better condition for allowance, they should be directed to the undersigned attorney.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 07-1180.

Dated: 01/09/2009

Respectfully submitted,

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